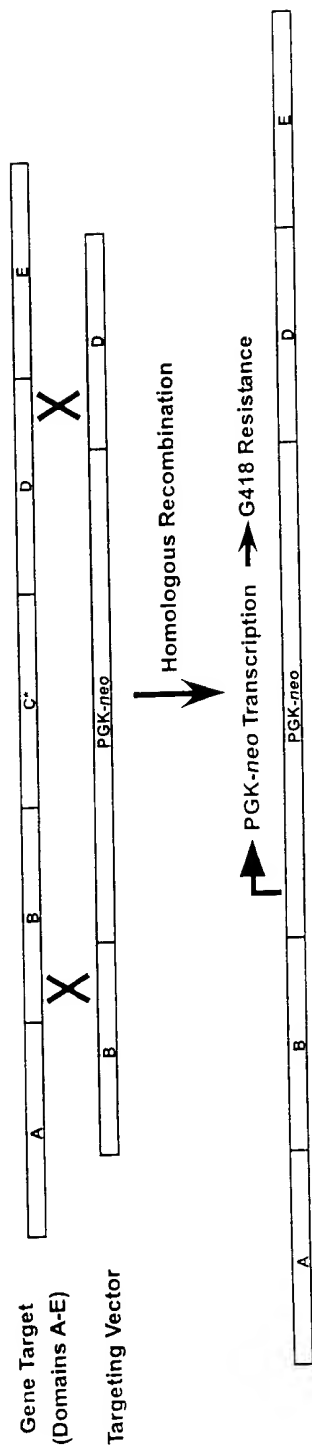
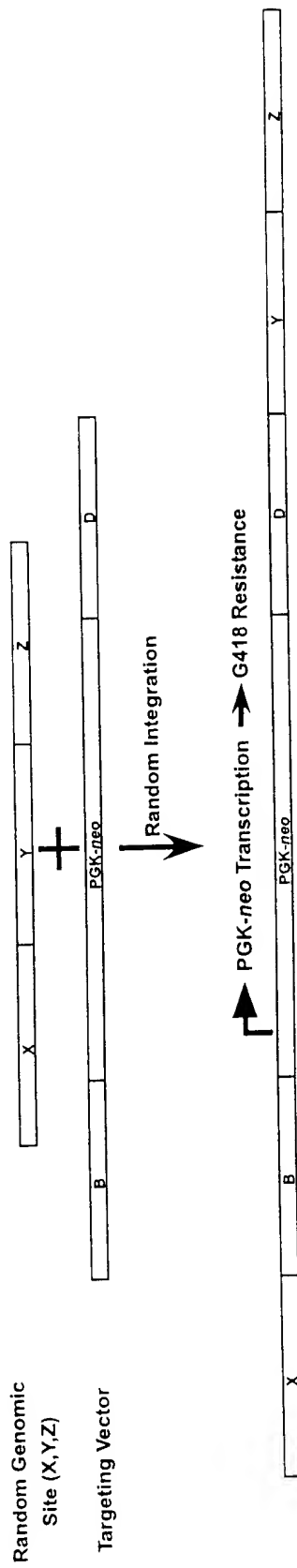


Figure 1

A. Homologous Recombination: G418 Resistance. Targeting Vector Flanked by "A" and "E"



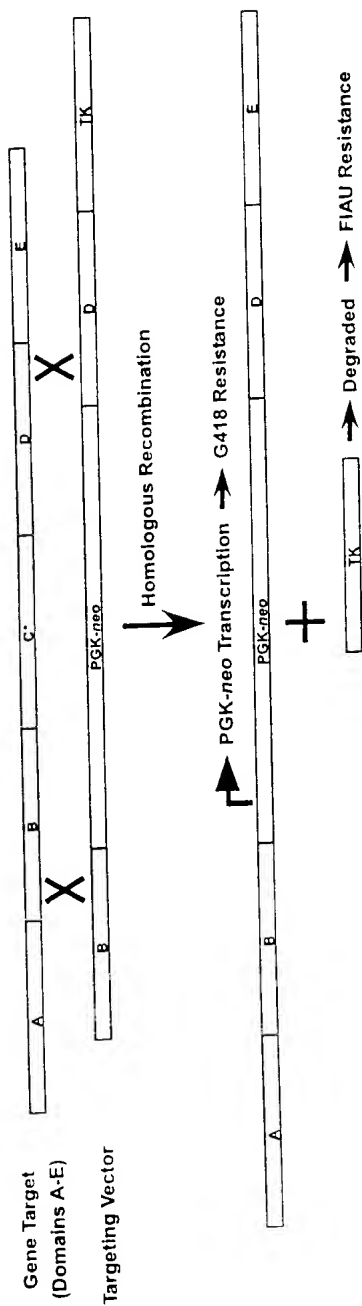
B. Random Integration: G418 Resistance. Targeting Vector Flanked by "X" and "Y"



DISTINGUISH EVENTS BY SCREENING MOLECULARLY (PCR & SOUTHERN)

Figure 2

A. Homologous Recombination: G418 Resistance + FIAU Resistance



B. Random Integration: G418 Resistance + FIAU Sensitivity

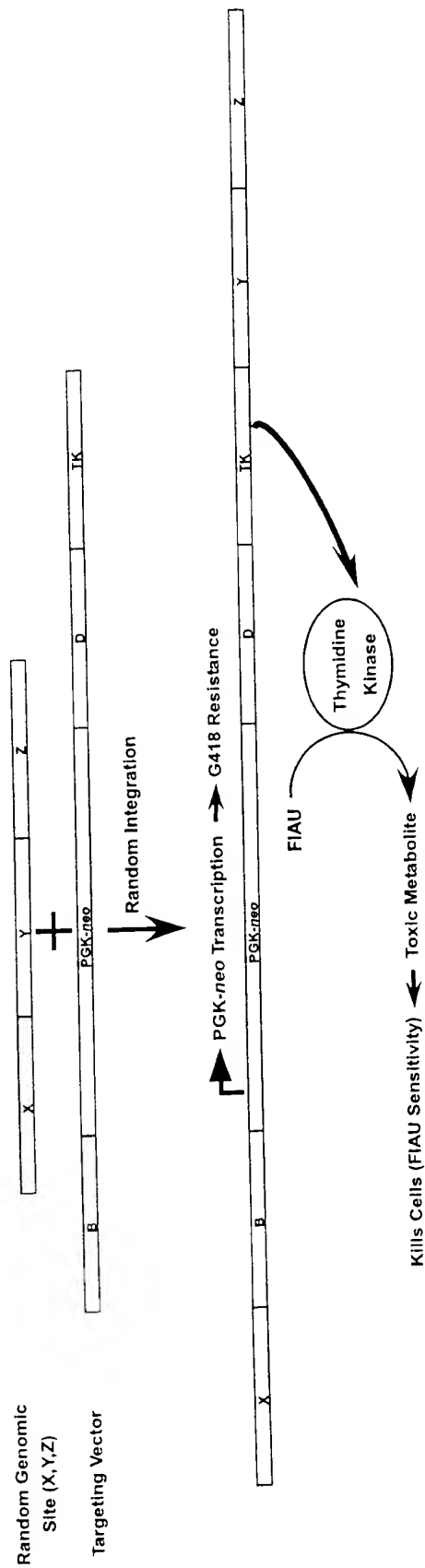


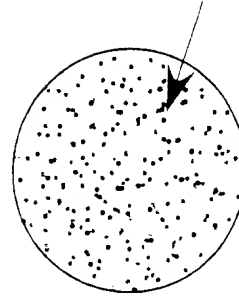
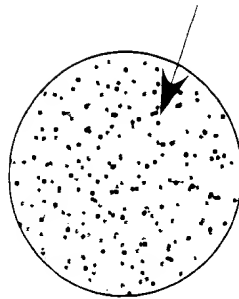
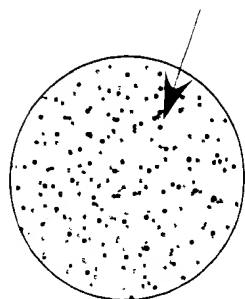
Figure 3

TRANSFECTION

A.

B.

C.

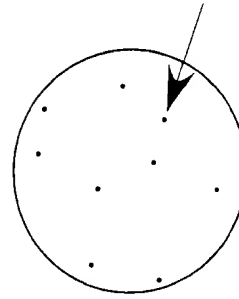
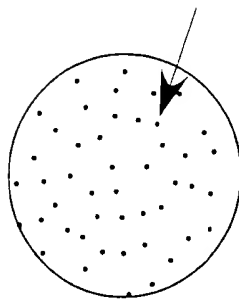
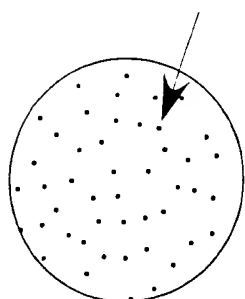


+ G418:
Select for Tf'd Cells

+ G418:
Select for Tf'd Cells

+ G418:
Select for Tf'd Cells &
Homologous Events

POSITIVE
SELECTION

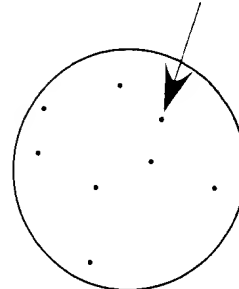


NEGATIVE
SELECTION

No Negative Selection

+ FIAU:
Select Against Some
Random Events

No Negative Selection



Screen Large
of Cells

Screen Small
of Cells

Screen Small
of Cells



SCREENING

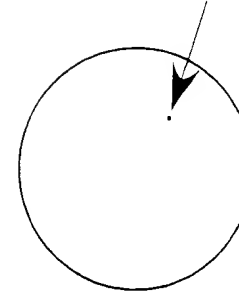
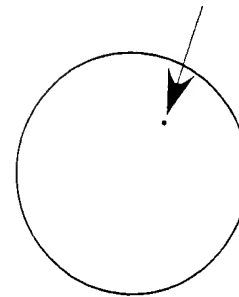
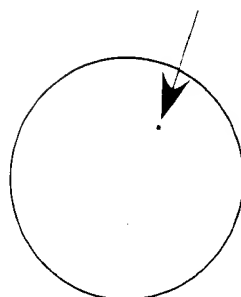
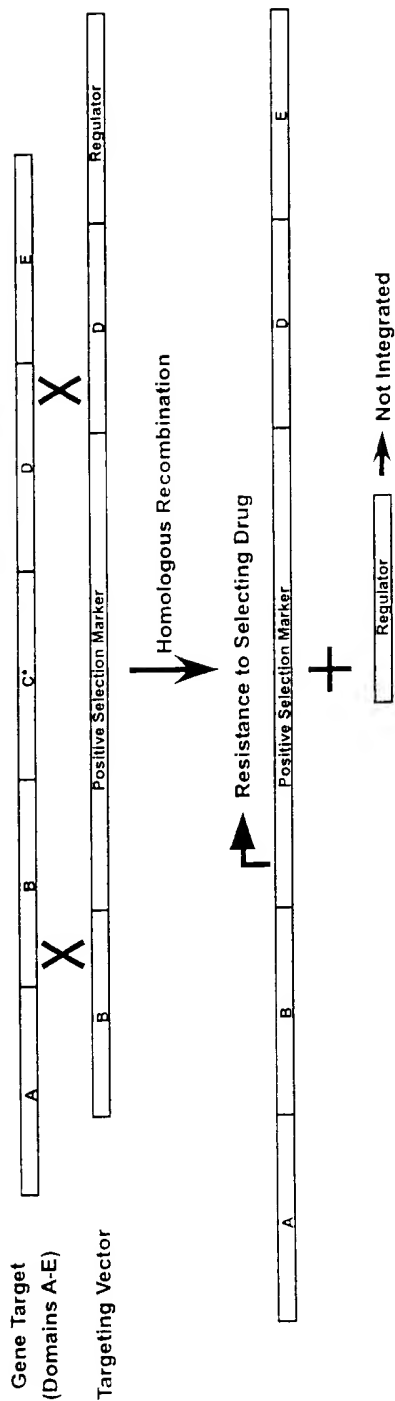


Figure 4

A. Homologous Recombination: Resistance to Selecting Drug



B. Random Integration: Sensitivity to Selecting Drug

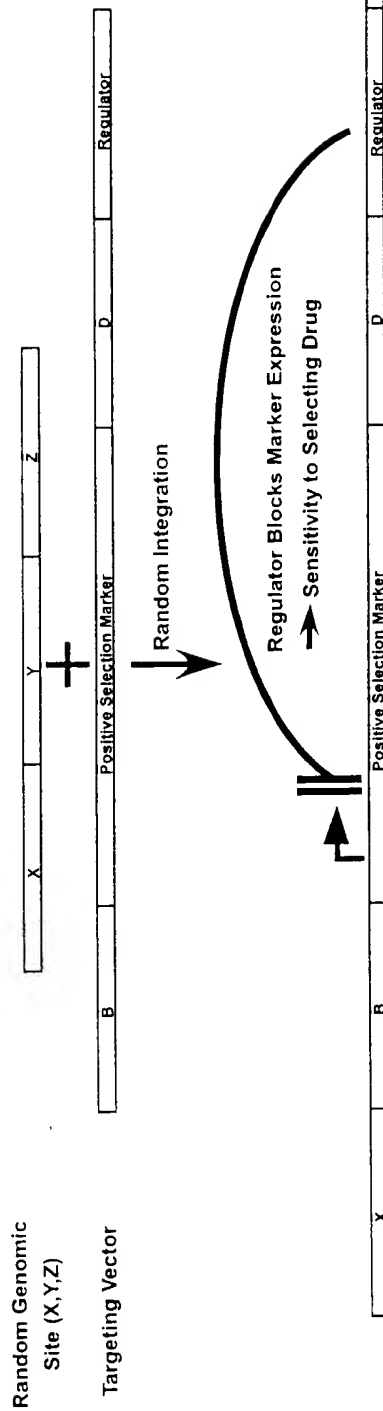


Figure 5

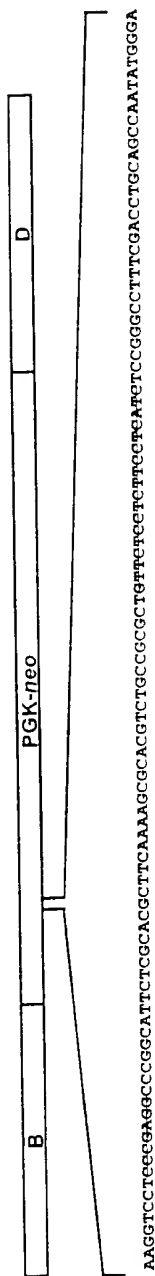
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TTTTTTT

Figure 6A

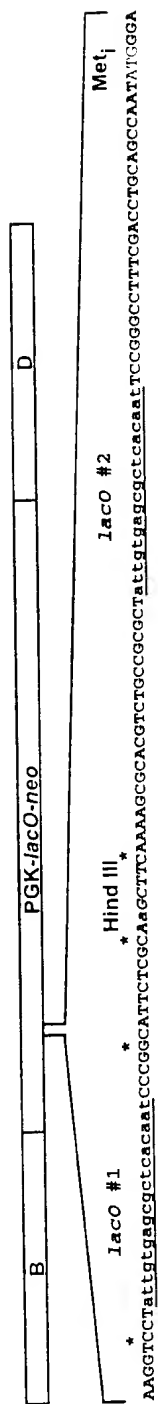
B. Gene Target
(Domains A-E)



C. Targeting Vector:
PGK-*neo*



D. Targeting Vector:
PGK-*lacO-neo*



E. Targeting Vector:
PGK-*lacO-neo*
+ NLS-*lacI*

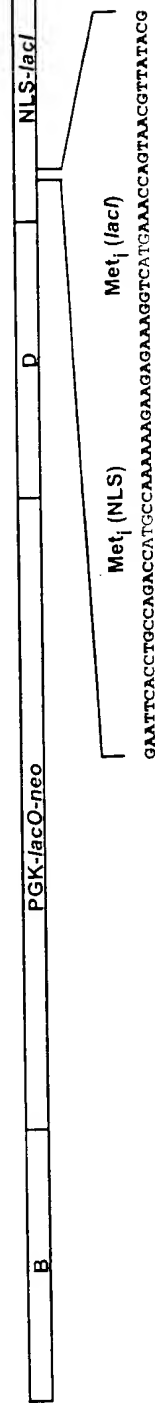
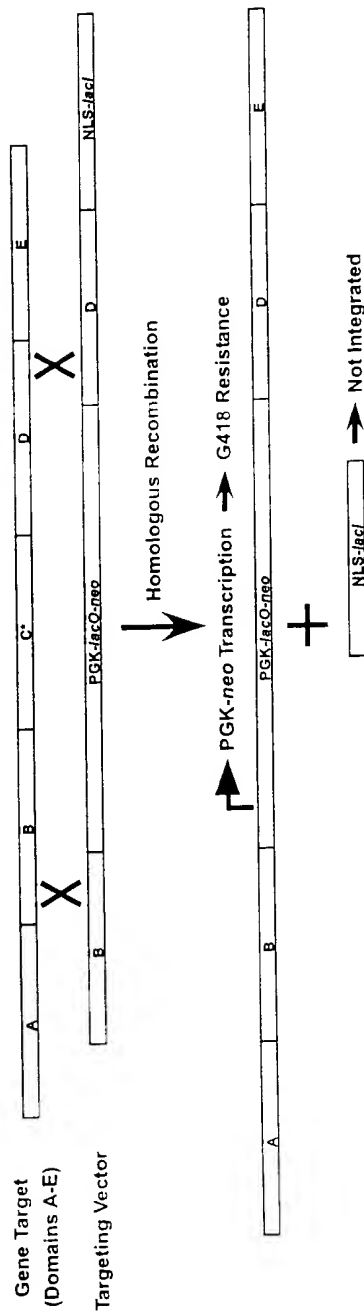


Figure 6 B-E

Homologous Recombination: G418 Resistance



Random Integration: G418 Sensitivity

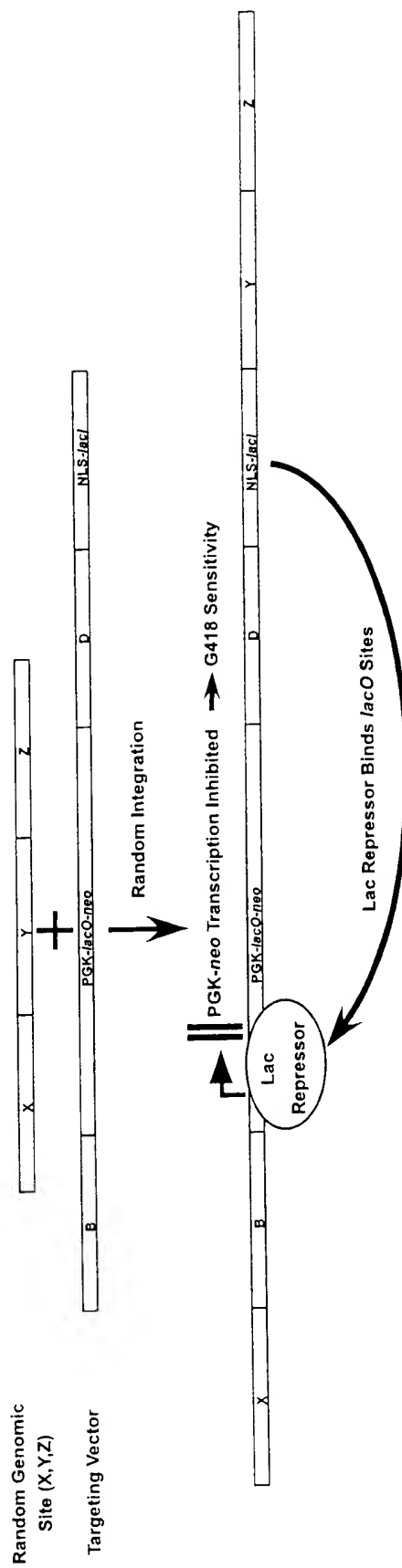


Figure 7

<u>Oligo #</u>	<u>Sequence (5' to 3')</u>
10164	CGGAATTCACCTGCCAGACCATGCCAAAAAAGAAGAGAAAGGTCATGAAACCAGTAACGTTATACG
10165	CGGAATTCTCACTGCCCGCTTTCCAGTCG
10218	GCATTCTCGCAAGCTTCAAAAGCGCACGTCTGCCGCGGTATTGTGAGCGCTCACAATTCCGGGCGCTTTCCGACCTG
9959	TCATCAATTTCTGCAGAC
10219	TGCGCTTTTGAAGCTTGCGGAGAATGCCGGSATTGTGAGCGCTCACAATAGGACCTTCCGCGCCCGCC
4201	CAGGAAACAGCTATGAC

Figure 8

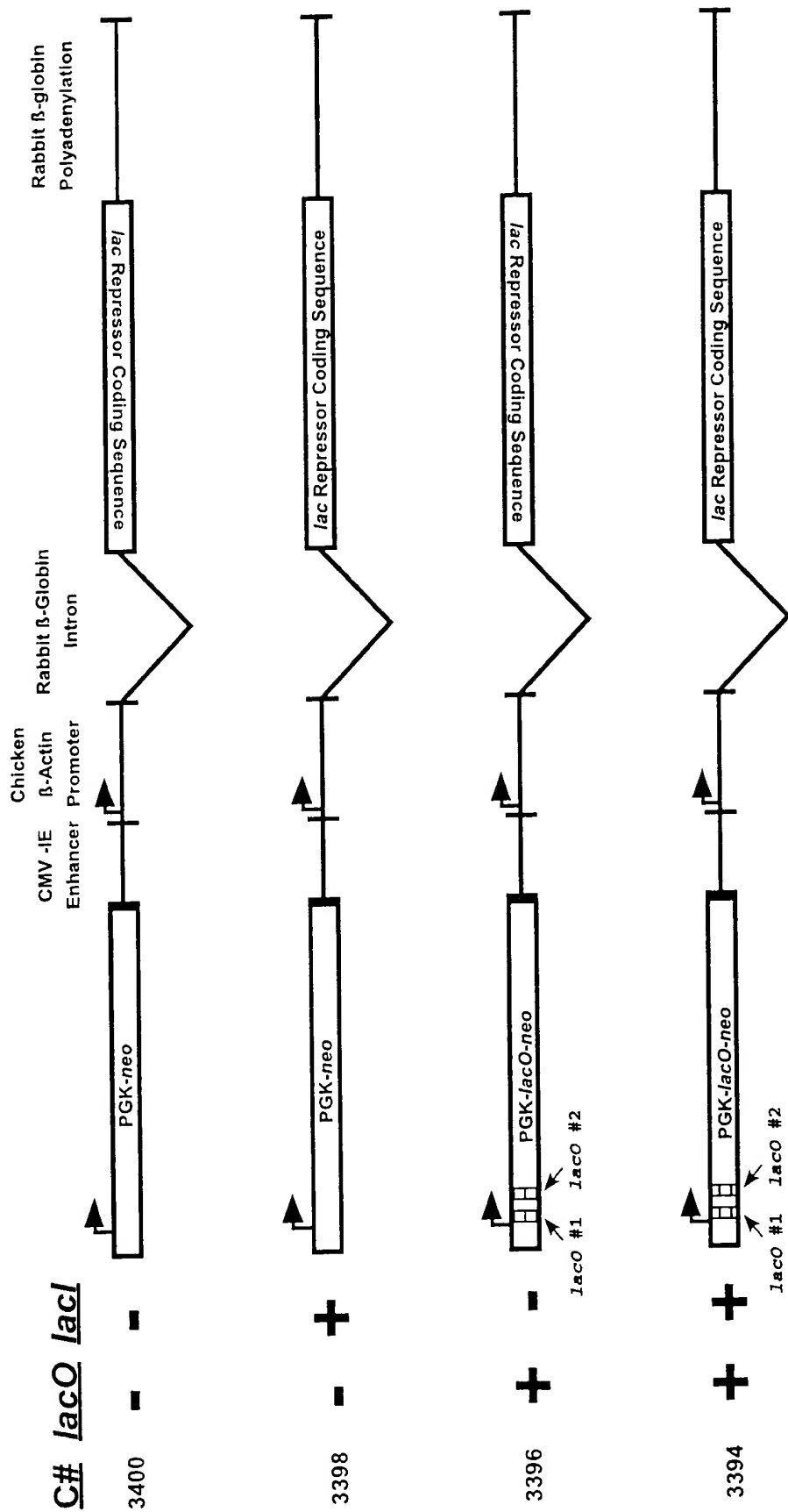


Figure 9

lacO + *lacI* REDUCE RANDOM INTEGRATION EVENTS

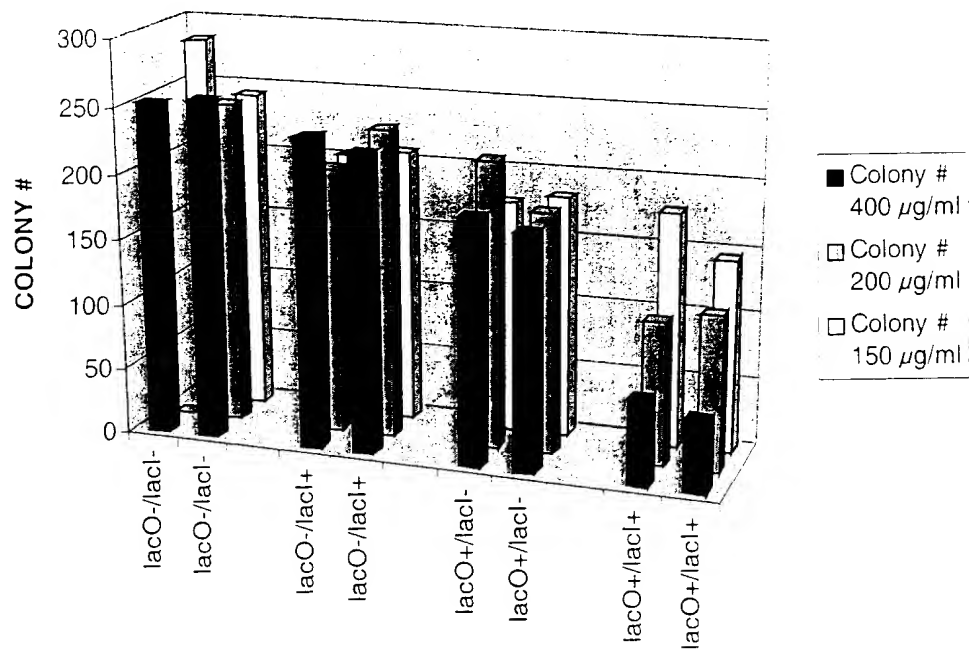


Figure 10

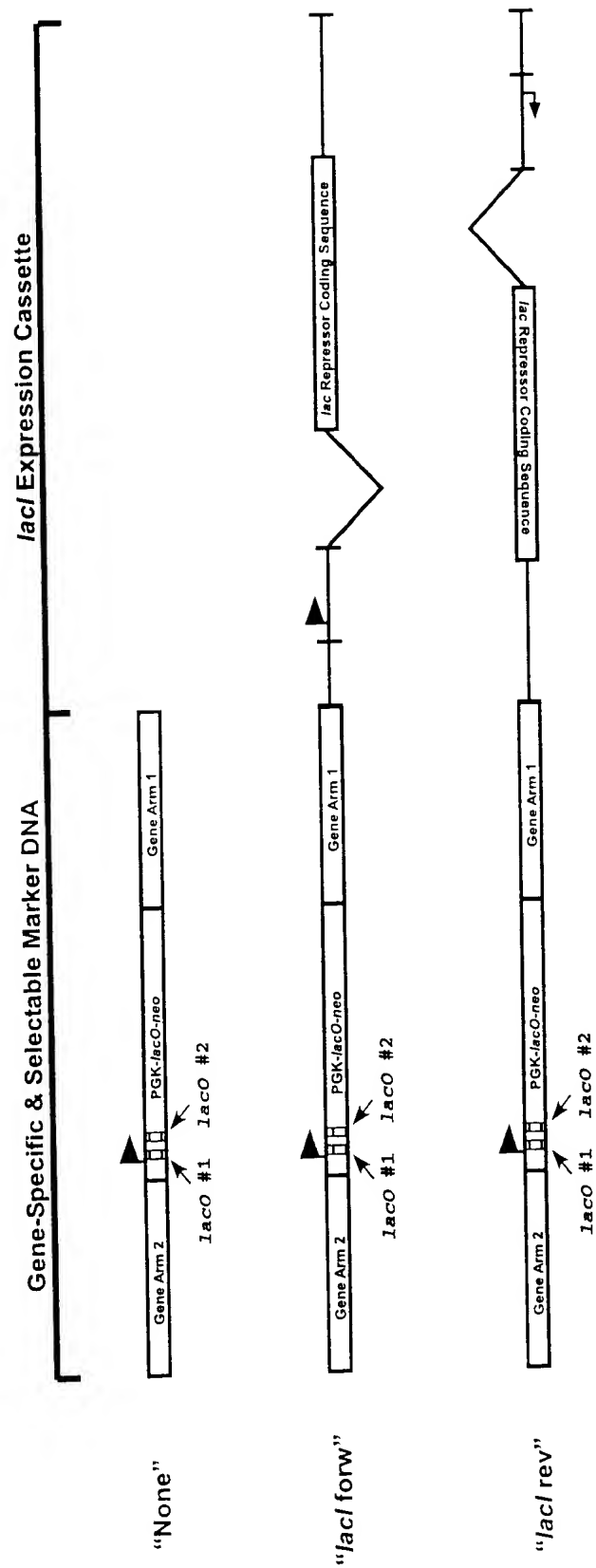


Figure 11

Homologous Recombinant Recovery Rate

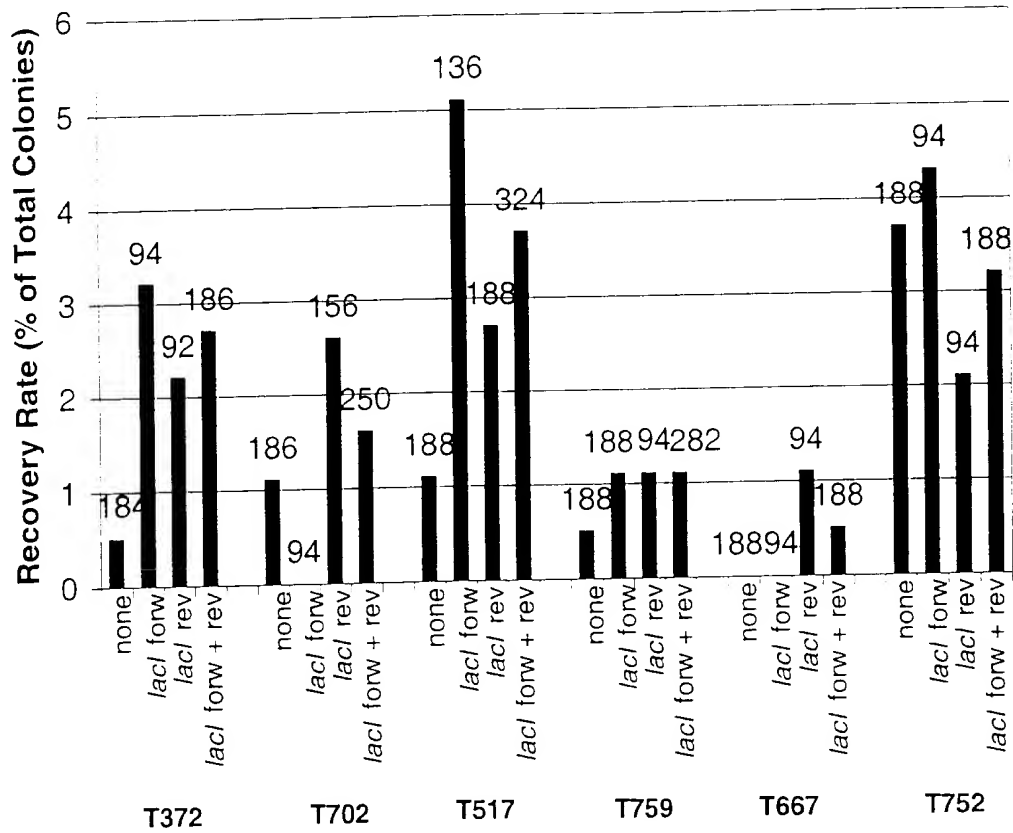


Figure 12

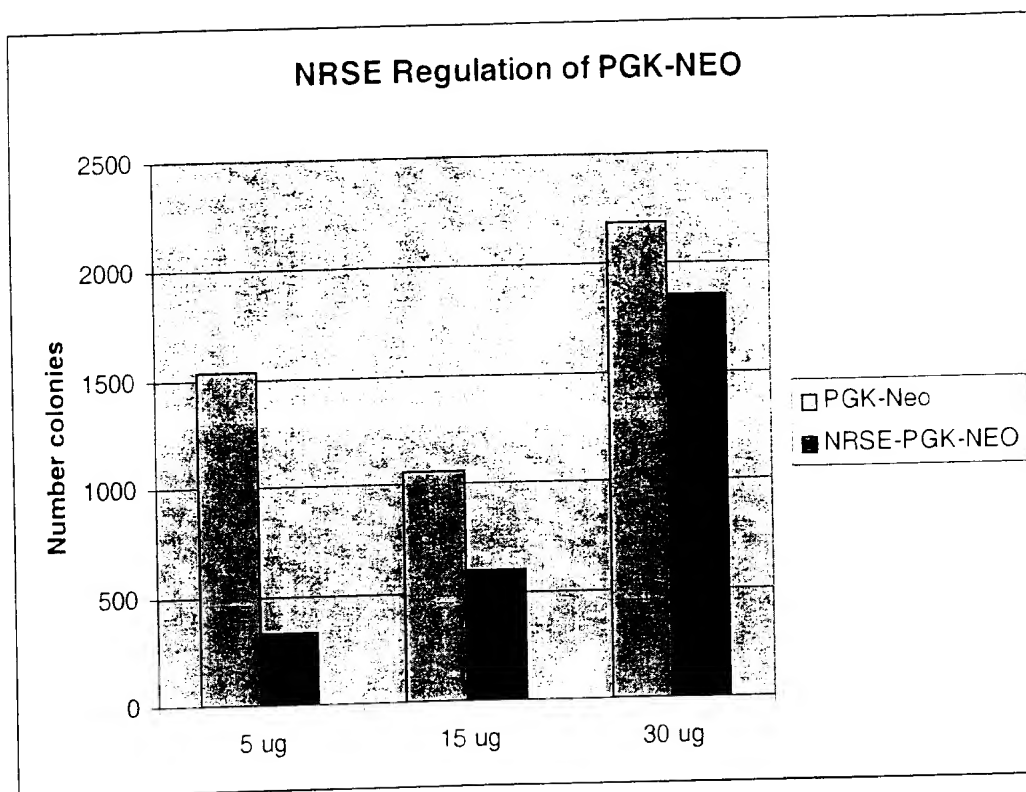


FIGURE 13

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Fig. 14B